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Adiponectin Deficiency Impairs Maternal Metabolic Adaptation to Pregnancy in Mice

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Hypoadiponectinemia has been widely observed in patients with gestational diabetes mellitus (GDM). To investigate the causal role of hypoadiponectinemia in GDM, adiponectin gene knockout (*Adipoq*$^{-/-}$) and wild-type (WT) mice were crossed to produce pregnant mouse models with or without adiponectin deficiency. Adenoviral vector–mediated in vivo transduction was used to reconstitute adiponectin during late pregnancy. Results showed that *Adipoq*$^{-/-}$ dams developed glucose intolerance and hyperlipidemia in late pregnancy. Increased fetal body weight was detected in *Adipoq*$^{-/-}$ dams. Adiponectin reconstitution abolished these metabolic defects in *Adipoq*$^{-/-}$ dams. Hepatic glucose and triglyceride production rates of *Adipoq*$^{-/-}$ dams were significantly higher than those of WT dams. Robustly enhanced lipolysis was found in gonadal fat of *Adipoq*$^{-/-}$ dams. Interestingly, similar levels of insulin-induced glucose disposal and insulin signaling in metabolically active tissues in *Adipoq*$^{-/-}$ and WT dams indicated that maternal adiponectin deficiency does not reduce insulin sensitivity. However, remarkably decreased serum insulin concentrations were observed in *Adipoq*$^{-/-}$ dams. Furthermore, β-cell mass, but not glucose-stimulated insulin release, in *Adipoq*$^{-/-}$ dams was significantly reduced compared with WT dams. Together, these results demonstrate that adiponectin plays an important role in controlling maternal metabolic adaptation to pregnancy.

Gestational diabetes mellitus (GDM) is a metabolic complication of pregnancy. GDM affects not only pregnancy outcomes but also metabolism in later life of both the GDM mothers and their offspring (1–4). Insulin resistance and insulin insufficiency have been considered to be the main underlying mechanisms of GDM (5). However, the causes of insulin resistance and insulin insufficiency are still largely unclear, which impedes the development of preventive and therapeutic approaches to reducing GDM and its impact on enhancing obesity in the offspring.

Adiponectin is an adipocyte-secreted hormone that enhances insulin sensitivity and improves glucose metabolism. During pregnancy, adiponectin is expressed and circulated in maternal and fetal compartments separately (6,7). In humans, maternal blood adiponectin concentrations decline during late pregnancy, which has been proposed as a potential underlying mechanism for insulin resistance that develops during late gestation in normal pregnancies (8). Obesity is a key risk factor for GDM, and obesity decreases adiponectin gene expression. Significantly low levels of blood adiponectin (hypoadiponectinemia) have been widely observed in pregnant women with obesity and/or GDM (9). Most importantly, hypoadiponectinemia before pregnancy and during the first and second trimesters is a major risk factor for GDM (10–13). Therefore, results from these human studies strongly suggest that hypoadiponectinemia may underlie GDM. However, there is no experimental evidence to support this hypothesis or to clarify how hypoadiponectinemia contributes to the metabolic defects in GDM.

Using genetic mouse models, the current study investigated the regulatory effects of adiponectin on maternal glucose and lipid metabolism during pregnancy. Our study found that, unlike virgin *Adipoq*$^{-/-}$ mice, pregnant *Adipoq*$^{-/-}$ mice spontaneously developed glucose

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intolerance and hyperlipidemia during late pregnancy. Significantly increased body weight and blood glucose levels were observed in fetuses from *Adipoq*$^{-/-}$ dams. After delivery, blood glucose and lipid profiles of *Adipoq*$^{-/-}$ dams were restored to the levels of wild-type (WT) mice. Remarkably increased hepatic glucose and triglyceride (TG) production rates and enhanced lipolysis of white adipose tissue (WAT) were detected in pregnant *Adipoq*$^{-/-}$ dams. Decreased serum insulin concentrations and β-cell mass indicated insulin insufficiency in pregnant *Adipoq*$^{-/-}$ mice. Furthermore, in vivo adiponectin reconstitution attenuated adiponectin deficiency–induced glucose intolerance and restored fetal body weight. Together, these results indicate that hypoadiponectinemia may play a role in the development of GDM. Our study also revealed that pregnant *Adipoq*$^{-/-}$ mice provide a mouse model for GDM study.

RESEARCH DESIGN AND METHODS

Materials

Glucose, glucose oxidase, BRL37344, DMEM, and RPMI 1640 medium were from Sigma-Aldrich (St. Louis, MO). Antibodies against Akt, phospho-Akt (Ser473), hormone-sensitive lipase (HSL), phosphor-HSL (Ser660), adipose triglyceride lipase (ATGL), peroxisome proliferator–activated receptor γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), Ki-67, AMPK, and phosphor-AMPK (Thr172) were from Cell Signaling Technology (Danvers, MA). Antibody against mouse adiponectin was from R&D Systems (Minneapolis, MN). Anti-GAPDH, PEPCK, glucose-6-phosphatase (G6Pase), and horseradish peroxidase–linked secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The lipoprotein lipase (LPL) activity assay kit was from Cell Biolabs (San Diego, CA). Free fatty acid (FFA) and TG assay kits ware purchased from Wako Diagnostics (Richmond, VA). NuPAGE gels, SuperScript III reverse transcriptase, and oligo(dT)$_{12–18}$ primer were from Invitrogen (Carlsbad, CA). The mouse diabetes multiplex assay kit was from Bio-Rad (Hercules, CA).

Experimental Animals

Adipoq$^{-/-}$ mice were created as previously described (14) and had been back crossed to C57BL/6. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Ten- to twelve-week-old nulliparous female mice were randomly selected for mating. *Adipoq*$^{-/-}$ female mice were mated with WT males or WT female mice mated with *Adipoq*$^{-/-}$ males. This cross breeding produced all fetuses that were *Adipoq*$^{-/+}$. For the studies of maternal metabolic adaptation during pregnancy, C57BL/6 mice were mated. Pregnancy was determined by the presence of a vaginal plug and was assigned the embryonic day (E) 0.5. To reconstitute adiponectin, 1×10^6 pfu of purified adenoviral vectors encoding adiponectin (Ad-*Adipoq*) or green fluorescent protein (Ad-*gfp*) were injected through the tail vein into *Adipoq*$^{-/-}$ dams at E15.5 (15,16). Glucose tolerance tests (GTTs) were performed at E16.5 after 6-h fasting with intraperitoneal injection of glucose (2 g/kg of body weight). Maternal tissues, placentas, and fetuses were collected at indicated gestational days through cesarean section. Except for some studies with fasted mice (see detail in the legends of Figs. 2*D* and 5*B*), tissue samples were collected when the mother was in the fed state. Body composition was determined by using EchoMRI System (Houston, TX). Experiments using mouse models were performed under the Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval from the University of California San Diego Animal Care and Use Committee.

Hyperinsulinemic-Euglycemic Clamp Assay

As we previously described (17), 4 days after surgery, the hyperinsulinemic-euglycemic clamp experiments began with a constant infusion of D-3-^{3}H-glucose (5 μCi/h; DuPont NEN, Boston, MA). Hepatic glucose production (HGP) and glucose disappearance rate (GDR) were calculated in the basal state and during the steady-state phase of the clamp.

Hepatic TG Production

Hepatic TG secretion rates were measured at E18.5 after inhibiting endogenous LPL activity by intravenous injection of Poloxamer-407 (1,000 mg/kg; BASF, Mount Olive, NJ), as we previously described (16). Mice were fasted for 4 h before injection. Blood TG concentrations were measured using a Wako kit.

WAT Lipolysis

WAT explants were collected at E18.5 for measuring lipolysis (18). Approximately 20 mg of gonadal adipose tissue explants were incubated in DMEM with 0.5% fatty acid–free BSA. β3-Adrenergic receptor agonist BRL37344 was added into medium at 50 ng/mL. Medium samples were collected at 30-, 60-, 120-, and 180-min intervals after adding BRL. The levels of FFA and free glycerol were measured and normalized to the weight of adipose explants.

Plasmid Constructs and Generation of Adenovirus Vectors

Adenoviruses encoding mouse adiponectin or GFP were created using the pAd/CMV/V5-Dest vector (Invitrogen). Construction and purification of the viral vectors were previously described (16).

Immunohistochemistry and β-Islet Morphometric Analysis

Pancreatic biopsy samples were fixed in 10% neutral-buffered formalin, processed, and paraffin embedded. Tissue sections were blocked with 2% H_2O_2 in PBS and then heated in 0.1 mol/L pH 6.0 citrate buffer for 15 min at 95°C for antigen retrieval. After second round blocking, immunostaining of insulin was done using anti-insulin primary antibody (10 μg/mL) or rabbit serum (for negative control) for 4 h. The sections were visualized using 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA) at room temperature for 1.5 min and counterstained with hematoxylin. β-Islet and

pancreas areas were measured using insulin-stained series of sections with the Leica SlidePath software. The percentage of β-islet area in the pancreas was determined by dividing the area of insulin-positive cells in one section by the area of the section. The β-cell mass was calculated by multiplying the pancreas weight by the percentage of islet area (19). Immunofluorescence was performed using sheep anti-insulin or rabbit antiglucagon primary antibody with paraffin-embedded pancreas sections.

β-Islet Isolation and Insulin Secretion

Pancreases were collected at E18.5. Islets were isolated by collagenase digestion and differential centrifugation through Ficol gradients using a protocol previously described (19). After overnight culture in RPMI with 5 mmol/L glucose, similar size islets were handpicked and incubated for 1 h in Krebs-Ringer medium with 2 mmol/L glucose. The islets were stimulated by adding glucose (20 mmol/L) or KCl (30 mmol/L) for 1 h. Medium was collected for insulin measurement.

Western Blot and Real-time PCR Assays

Protein samples were extracted from fat, livers, or skeletal muscle and separated using NuPAGE gels. Proteins were blotted with the indicated antibodies (see details in figure legends). The bands from Western blots were quantified using Quantity One software (Bio-Rad). Total RNA was prepared from tissues or cells using Trizol according to the manufacturer's protocol. cDNA was synthesized using SuperScript III Reverse Transcriptase and oligo$(dT)_{12–18}$ primer. Real-time PCR was performed using an Mx3000P Real-Time PCR System (Stratagene, San Diego, CA) and specific primers (Table 1). The levels of PCR product were calculated from standard curves established from each primer pair. Expression data were normalized to the amount of 18S rRNA.

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analyses were performed using the Student *t* test or ANOVA, followed by Bonferroni posttests using Prism software. Differences were considered significant at $P < 0.05$.

RESULTS

Maternal Metabolic Adaptation in C57BL/6 Mice

Although mouse has become the premier mammalian model in biomedical research due to its genetic and physiological similarities to humans and easy manipulation of the genome, information about maternal metabolic adaptation in mice is very limited. Using C57BL/6 mice, our study showed that pregnant mice gained significant amounts of body weight (data not shown). The increase of the dam's body weight was mainly manifested through increasing liver and fat tissue mass (Fig. 1*A* and *B*). Similar to humans (20), the dam's blood glucose concentrations were significantly decreased in late pregnancy (Fig. 1*C*). The decrease of maternal blood glucose may be due to expansion of blood volume and the steadily increasing utilization rates of glucose by the uteroplacenta and fetus during late pregnancy. In contrast, maternal blood TG, FFA, and insulin levels were robustly elevated during pregnancy (Fig. 1*D–F*). Two weeks after delivery, most of these metabolic phenotypes, including blood glucose, TG, FFA, and insulin, recovered (Fig. 1*C–F*).

We also characterized pregnancy-induced WAT expansion. Analyzing gonadal fat histology (inguinal fat was not analyzed because of its mixture with fat and the mammary gland), we found that pregnancy increases maternal white adipocyte size (Fig. 1*G*) but not cell number (data not shown). A remarkable decrease in phosphorylation of HSL and protein levels of ATGL were found in gonadal fat (Fig. 1*H* and *I*). However, despite increased fat tissue mass, expression of the adipogenic transcription factors PPARγ and C/EBPα was reduced during pregnancy (Fig. 1*H* and *I*). These results indicate that pregnancy increases maternal WAT mass mainly by increasing lipid accumulation but not recruitment of new adipocytes. These results also indicate that WAT lipolysis is significantly suppressed during normal mouse pregnancy, which is similar to that in early and midpregnancy in humans. Also similar to pregnant women (8), significantly decreased adiponectin was observed in blood (data not shown) and WAT (Fig. 1*H* and *I*) of pregnant mice from E14.5 to the end of gestation.

Using hyperinsulinemic-euglycemic clamps at E16.5, our study found that insulin-stimulated GDR was significantly reduced (Fig. 1*J*) but basal HGP rates were significantly increased in pregnant mice (Fig. 1*K*). However, insulin-suppressed HGP reduction rates were similar between pregnant and nonpregnant mice (Fig. 1*L*), in line with human and dog studies (21–23). In addition, phosphorylation of AKT was significantly decreased in both gonadal fat and skeletal muscle (Fig. 1*H*, *I*, *M*, and *N*),

Table 1—Sequences for real-time PCR primers

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
18S rRNA	CGAAAGCATTTGCCAAGAAT	AGTCGGCATCGTTTATGGTC
Dgat1	TCGTGGTATCCTGAATTGGTG	AGGTTCTCTAAAAATAACCTTGCATT
Fasn	ACTCCACAGGTGGGAACAAG	CCCTTGATGAAGAGGGATCA
Srebp1c	GGTTTTGAACGACATCGAAGA	CGGGAAGTCACTGTCTTGGT
Vldlr	CCTATAACTAGGTCTTTGCAGATATGG	GAGCCCCTGAAGGAATGCC
Mttp	GCCCAACGTACTTCTAATTTATGG	TGCTGGCCAACACGTCTA

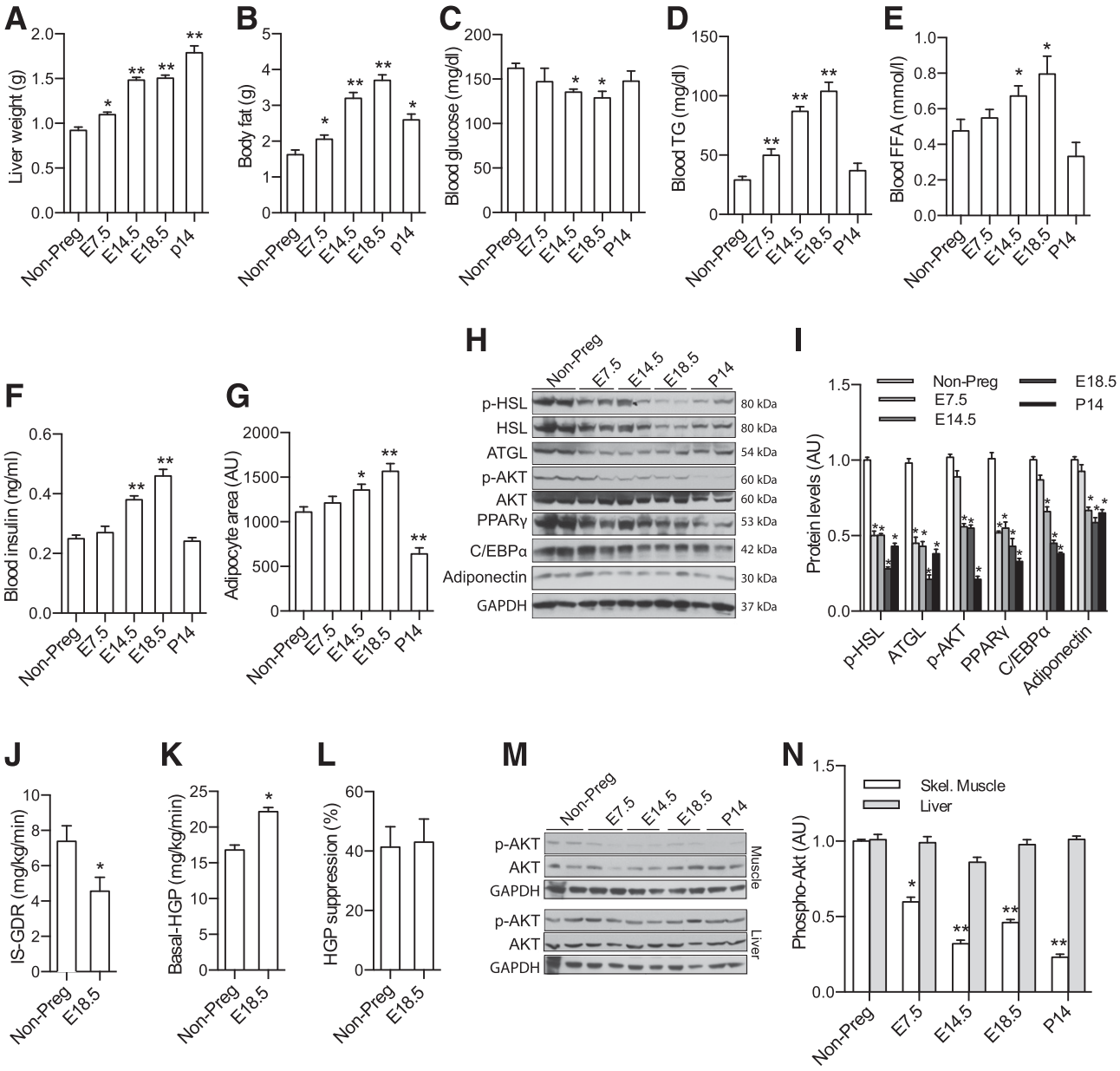

Figure 1—Maternal metabolic adaptation in mice. Nulliparous C57BL/6 mice (10–12 weeks old) were used. Pregnancy was determined by the presence of a vaginal plug and assigned as E0.5. Tissue samples were collected at fed state at indicated gestational or postpartum (P) 14 days. Increased maternal liver (*A*) and body fat (*B*) were observed during pregnancy and during lactation. Serum glucose (*C*), TG (*D*), FFA (*E*), and insulin (*F*) concentrations were measured using commercial kits. Adipocyte areas were analyzed using ImageJ software with hematoxylin-eosin–stained gonadal fat sections (*G*). Phosphorylation and protein levels were determined by Western blotting using proteins from gonadal fat (*H*), skeletal muscle (*M*, top), and liver (*M*, bottom). Western blotting of fat (*I*) and skeletal muscle and liver (*N*) were quantified by measuring image densities. Insulin-stimulated GDR (IS-GDR) (*J*), basal HGP (*K*), and insulin-suppressed HGP reduction (*L*) were measured using hyperinsulinemic-euglycemic clamping at E18.5. Data are presented as mean ± SEM; n = 8–10. $^*P < 0.05$ and $^{**}P < 0.01$ vs. Non-Preg (nonpregnant). AU, arbitrary units.

but not in the liver (Fig. 1*M* and *N*). These results indicate that pregnancy reduces insulin sensitivity in WAT and skeletal muscle and increases basal HGP in mice.

Adiponectin Deficiency Increases Maternal Blood Glucose, TG, and FFA Concentrations and Fetal Weight

We created adiponectin-deficient and control pregnant mouse models by crossing *Adipoq*$^{-/-}$ with WT mice. This breeding scheme produced *Adipoq*$^{-/+}$ fetuses, which avoided difference in fetal adiponectin. As we recently reported (24), there was no difference in pregravid body weights, adiposity, or litter size between *Adipoq*$^{-/-}$ and WT dams (data not shown). Surprisingly, blood glucose, FFA, and TG concentrations in *Adipoq*$^{-/-}$ dams were significantly higher than those of WT dams at E18.5 (Fig. 2*A*–*C*).

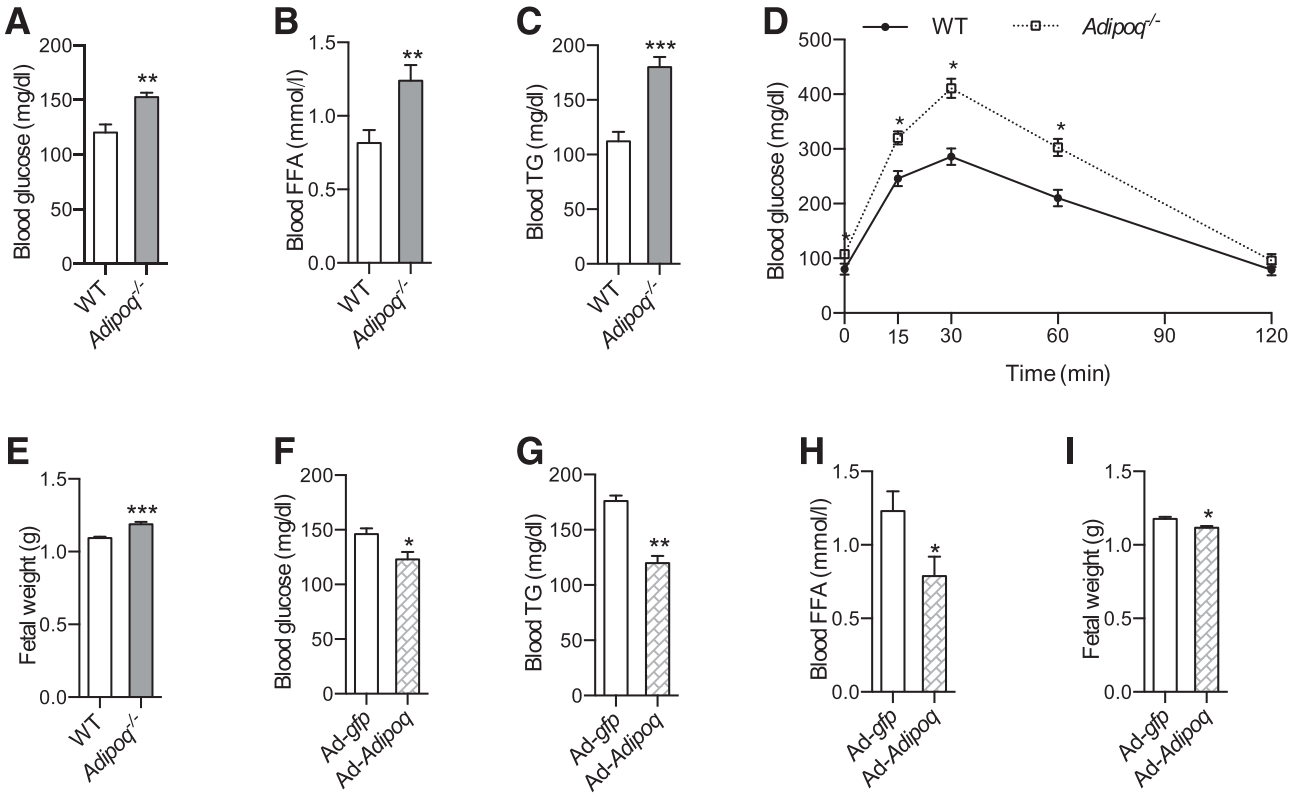

Figure 2—Adiponectin deficiency induces glucose intolerance and hyperlipidemia in mice. *Adipoq*$^{-/-}$ and WT mice were cross mated to produce *Adipoq*$^{-/-}$ and WT dams and all resultant fetuses were *Adipoq*$^{-/+}$. Blood glucose (*A*), FFA (*B*), and TG (*C*) concentrations and fetal weight (*E*) were measured using samples from fetuses sacrificed and autopsied at E18.5 of fed dams. GTT (*D*) was performed at E16.5 after 6-h fasting. Adiponectin was reconstituted by injecting Ad-*Adipoq* virus at E15.5 of *Adipoq*$^{-/-}$ dams (*F–I*). Ad-*gfp* was used as control. Dams' blood glucose (*F*), TG (*G*), and FFA (*H*) concentrations and fetal weight (*I*) were determined at E18.5 at fed state. Data are presented as mean ± SEM; n = 6–8. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. WT or Ad-*gfp*–treated *Adipoq*$^{-/-}$ dams.

Furthermore, glucose intolerance was detected in *Adipoq*$^{-/-}$ dams, including significantly increased fasting blood glucose concentrations (Fig. 2*D*, E16.5). Fetal body weights of *Adipoq*$^{-/-}$ dams were significantly higher than those of fetuses from WT dams (Fig. 2*E*). We used an adenoviral vector–mediated in vivo gene transduction technique to reconstitute maternal adiponectin in some *Adipoq*$^{-/-}$ dams (15,16,25). Results showed that 3-day adiponectin reconstitution reduced maternal blood glucose, TG, and FFA concentrations and fetal body weight at E18.5 compared with Ad-*gfp*–treated *Adipoq*$^{-/-}$ dams (Fig. 2*F–I*). In addition, 4 weeks after delivery, blood glucose, TG, and FFA levels of *Adipoq*$^{-/-}$ dams were completely recovered (data not shown). These results indicate that adiponectin plays an important role in controlling maternal metabolic adaptation to pregnancy.

Adiponectin Deficiency Does Not Induce Insulin Resistance but Increases Hepatic Gluconeogenesis in Pregnant Mice

Decreased glucose utilization in maternal peripheral tissues and/or increased HGP might impair glucose tolerance in GDM (26,27). Using hyperinsulinemic-euglycemic clamp assays, our studies found that insulin-stimulated GDRs of *Adipoq*$^{-/-}$ dams were comparable to WT dams (Fig. 3*A*). Both basal and insulin-stimulated phosphorylation of AKT protein in metabolically active tissues, such as skeletal muscle, liver, and WAT, were similar to those of WT dams (Fig. 3*B*, quantified data not shown). These results indicate that adiponectin deficiency did not induce insulin resistance in pregnant mice. Similarly, intact insulin sensitivity has been observed in chow-fed *Adipoq*$^{-/-}$ male mice (14).

Despite the fact that the underlying mechanism is still under debate, the inhibitory effect of adiponectin on hepatic gluconeogenesis has been well documented (14,28–31). Similar to nonpregnant mice (14), our clamp assay found that basal HGP rates were significantly increased in *Adipoq*$^{-/-}$ dams (Fig. 3*C*). Expression levels of PEPCK and G6Pase, rate-limiting enzymes of gluconeogenesis, were significantly increased in the livers of *Adipoq*$^{-/-}$ dams (Fig. 3*D*). Interestingly, insulin-induced HGP reduction and phosphorylation of AMPK in livers were similar between *Adipoq*$^{-/-}$ and WT controls (Fig. 3*D* and *E*). These results indicate that adiponectin deficiency increases maternal hepatic gluconeogenesis via a mechanism independent of insulin and AMPK signal transduction.

Adiponectin Deficiency Increases Maternal WAT Lipolysis and Hepatic TG Production

Our studies showed that lipolysis in WAT was remarkably suppressed during normal pregnancy (Fig. 1*H* and *I*). In a

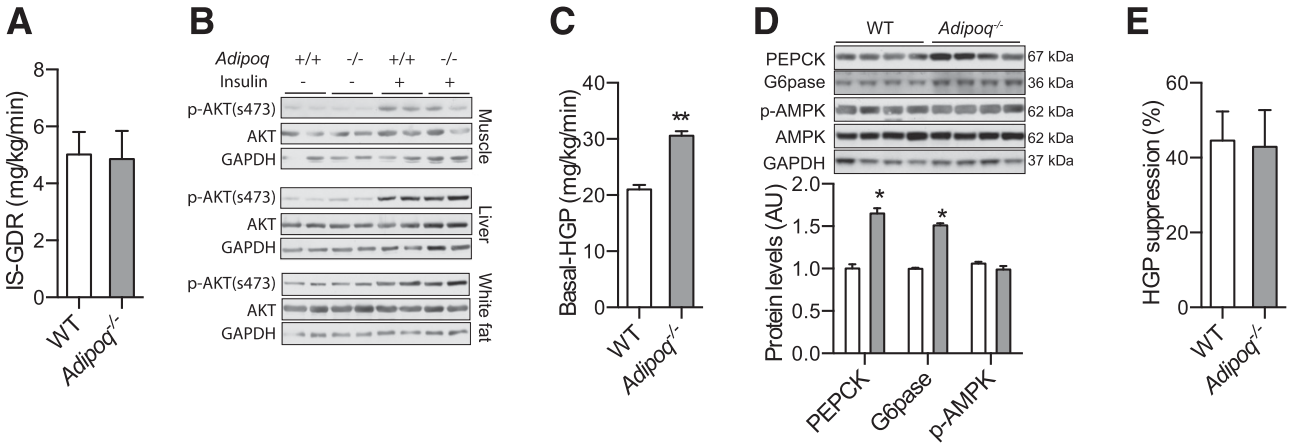

Figure 3—Adiponectin deficiency increases hepatic glucose production. *Adipoq*$^{-/-}$ and WT mice were cross mated to produce *Adipoq*$^{-/-}$ and WT dams. Insulin-stimulated GDR (IS-GDR) (*A*), HGP (*C*), and insulin-inhibited HGP (*E*) were measured by hyperinsulinemic-euglycemic clamp at E18.5. *B*: Phosphorylation of AKT was measured by Western blotting using tissues collected before and after 5-min insulin bolus stimulation. *D*: Protein levels were measured by Western blotting using liver samples after 6-h fasting. Data are presented as mean ± SEM; $n = 6$. *$P < 0.05$ and **$P < 0.01$ vs. WT dams. AU, arbitrary units.

previous study we demonstrated that adiponectin inhibits lipolysis in adipocytes (18). Therefore, in the present studies, we measured WAT lipolysis in *Adipoq*$^{-/-}$ dams. We found that phosphorylation of HSL was significantly increased in gonadal fat of *Adipoq*$^{-/-}$ dams (Fig. 4*A*). Protein levels of the rate-limiting enzyme ATGL, but not perilipin and CGI58 (coactivator of ATGL), were also significantly increased in gonadal fat of *Adipoq*$^{-/-}$ dams (Fig. 4*A*). Using gonadal fat explants, we found that both basal and β3-adrenergic agonist BRL37344–stimulated glycerol release rates were significantly increased in WAT of *Adipoq*$^{-/-}$ dams (Fig. 4*B*). Together, these results indicate that lipolysis of WAT was significantly enhanced in pregnant mice with adiponectin deficiency.

Remarkably increased blood TG concentrations were observed in *Adipoq*$^{-/-}$ dams (Fig. 2*C*). Using male mice, we had reported that adiponectin improves blood TG profile by enhancing LPL expression in skeletal muscle (16). Unlike male mice, LPL protein levels (Fig. 4*C*) and activity (Fig. 4*D*) in skeletal muscle were comparable between *Adipoq*$^{-/-}$ and WT dams at E18.5. However, hepatic TG secretion rates of *Adipoq*$^{-/-}$ dams were significantly higher than those of WT dams (Fig. 4*E*). These results indicate that maternal adiponectin deficiency increases hepatic TG production, which may be the main cause of hypertriglyceridemia in these pregnant mice. Furthermore, we also found that expression of *Dagt1* and *Mttp*, which are key genes for TG synthesis and VLDL-TG assembly, but not de novo lipogenic genes *Fasn* and *Srebp1c*, was significantly increased in livers of *Adipoq*$^{-/-}$ dams (Fig. 4*F*). Fatty acids used for VLDL assembly can originate from sources other than hepatic de novo fatty acid synthesis (32). Therefore, enhanced lipolysis of WAT should provide fatty acids for reesterification in hepatocytes and TG production.

Adiponectin Deficiency Induces Insulin Insufficiency in Pregnant Mice

Due to metabolic demand, there is a large compensatory β-cell expansion during pregnancy (33). A significant increase in maternal blood insulin concentrations (Fig. 1*F*) was observed in C57BL/6 dams. Interestingly, despite increased blood glucose concentrations in *Adipoq*$^{-/-}$ dams (Fig. 2*A* and *D*), their serum insulin concentrations were significantly lower than those of WT dams at fed state and during GTT (Fig. 5*A* and *B*). These results indicate that there is an insulin insufficiency in *Adipoq*$^{-/-}$ dams.

Compensatory expansion of β-cell mass during pregnancy occurs mainly through increased β-cell proliferation, cell size, and reduced apoptosis (33). We performed a morphometric analysis of the pancreases. β-Cell islets of *Adipoq*$^{-/-}$ dams were significantly smaller than those of WT dams (Fig. 5*C* and *D*). Quantitative analysis showed that β-cell mass of *Adipoq*$^{-/-}$ dams was significantly lower than that of WT controls (Fig. 5*E*). In contrast, adiponectin reconstitution significantly increased maternal blood insulin concentrations and β-cell mass in *Adipoq*$^{-/-}$ dams (Fig. 5*F* and *G*). We then studied glucose-stimulated insulin secretion using isolated islets. As shown in Fig. 5*H*, high concentrations of glucose or KCl stimulated similar levels of insulin release from islets from *Adipoq*$^{-/-}$ dams and WT controls, indicating that β-cells from *Adipoq*$^{-/-}$ dams were sensitive to glucose-stimulated insulin secretion. These results indicate that decreased β-cell mass is most likely responsible for the insulin insufficiency of *Adipoq*$^{-/-}$ dams.

DISCUSSION

Although hypoadiponectinemia before and during early pregnancy closely associates with the development of GDM in late pregnancy (10–13), the casual relationship

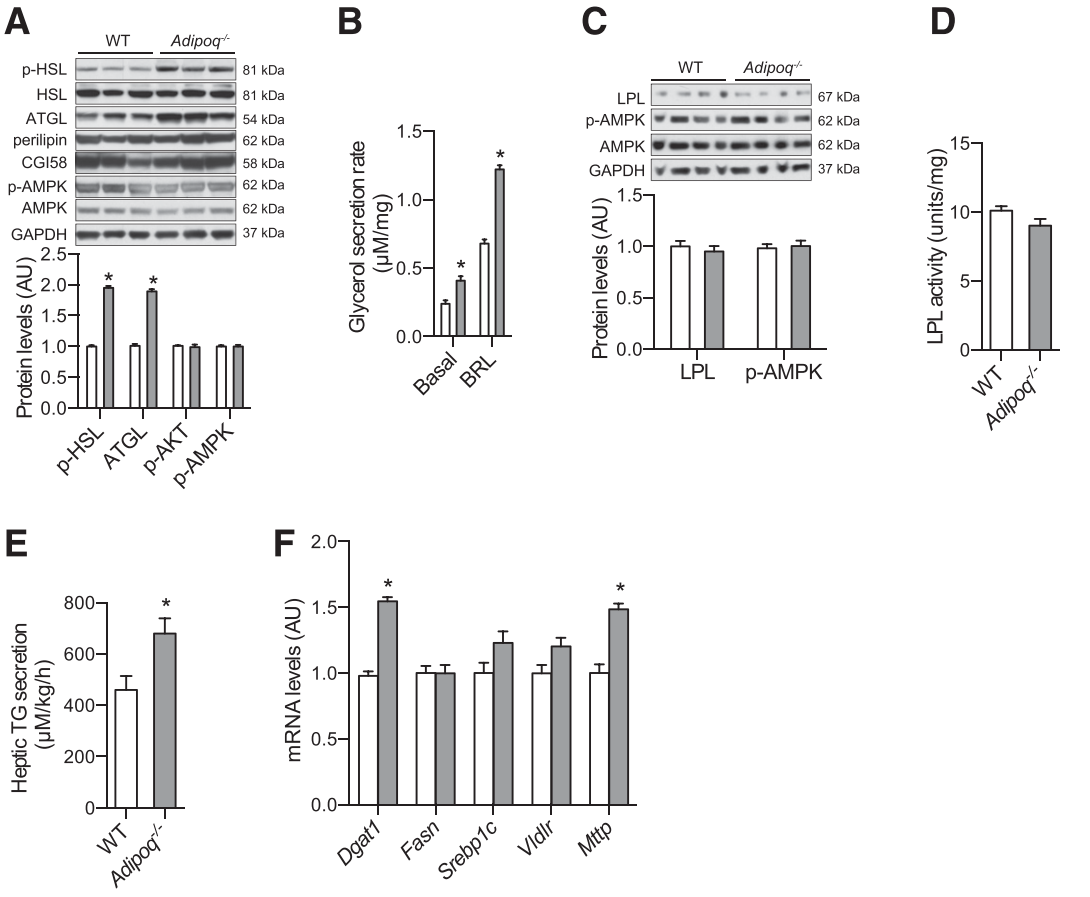

Figure 4—Adiponectin deficiency enhances lipolysis in WAT and hepatic TG production. Tissue samples were collected from *Adipoq*$^{-/-}$ and WT dams at E18.5. Phosphorylation and protein levels in the gonadal fat (*A*) and skeletal muscle (*C*) were measured by Western blotting. Lipolysis was studied by measuring glycerol release rates using gonadal explants at basal and after β3 ligand BRL stimulation (*B*). LPL activities were measured using skeletal muscle lysates (*D*). Hepatic TG production rates were measured after 4 h fasting and injection of LPL inhibitor (*E*). mRNA levels in the livers were measured by real-time PCR (*F*). Data are presented as mean ± SEM; $n = 8$. *$P < 0.05$ vs. WT. AU, arbitrary units.

between hypoadiponectinemia and GDM has not been experimentally verified. Using genetic mouse models, this study demonstrated that pregnant mice with adiponectin deficiency spontaneously developed glucose intolerance, hyperlipidemia, and fetal overgrowth. Furthermore, adiponectin reconstitution during late pregnancy restored maternal metabolism and fetal body weight. Together, these data demonstrate that adiponectin plays an important role in controlling maternal metabolic adaptation to pregnancy. Maternal adiponectin deficiency induces major metabolic defects of GDM in mice, including glucose intolerance, hyperlipidemia, and high fetal weight. This experimental evidence further enforces the idea that hypoadiponectinemia plays a potential role in the development of GDM in humans.

Three lines of *Adipoq*$^{-/-}$ mice have been created (14,34,35). Interestingly, without high-fat diet challenge, two lines (including the line used in this study) do not exhibit any peripheral insulin resistance or glucose intolerance (14,35). We have analyzed virgin *Adipoq*$^{-/-}$, *Adipoq*$^{-/+}$, and WT littermates from heterozygous matings and found that their blood glucose and insulin concentrations were comparable during a period from weaning to 1 year old (data not shown). We were surprised to observe glucose intolerance and hyperlipidemia in *Adipoq*$^{-/-}$ dams. Therefore, these results indicate that pregnancy induces *Adipoq*$^{-/-}$ mice to develop glucose intolerance and hyperlipidemia, which are the main characteristics of GDM. In addition, significantly increased fetal weight of *Adipoq*$^{-/-}$ dams resembles the macrosomia characteristics of GDM. Together, these observations indicate that pregnant *Adipoq*$^{-/-}$ mice provide a useful mouse model of GDM. It should be pointed out that a human study reported that hypoadiponectinemia during pregnancy predicts postpartum insulin resistance and β-cell dysfunction (36). After giving birth, women with GDM may still have metabolic defects and have a much higher risk for developing type 2 diabetes (2,3,37). However, our study found that 4 weeks after delivery, all metabolic alterations in *Adipoq*$^{-/-}$ dams were recovered, which indicates the difference between human GDM and this genetic mouse model. It is known that GDM is a multifactor disease (5,37). We speculate that adiponectin gene knockout resembles only

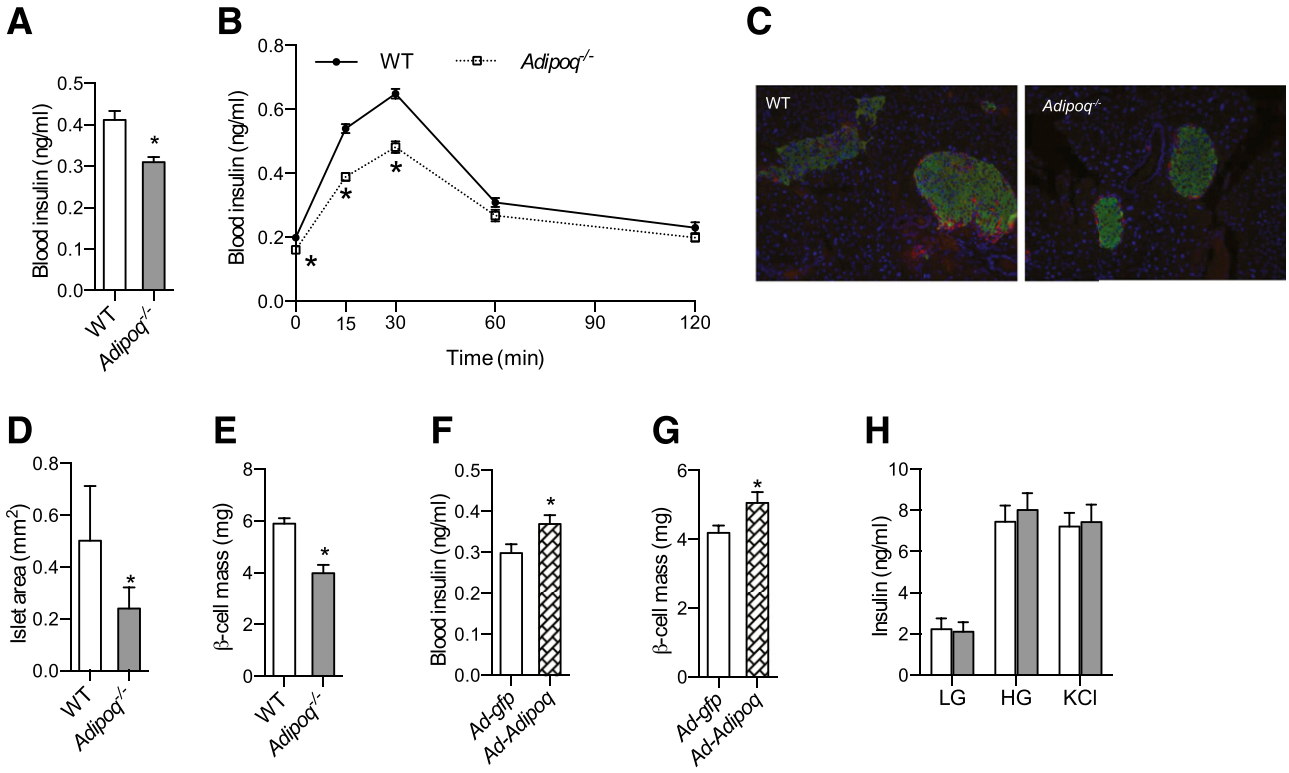

Figure 5—Adiponectin deficiency impairs compensatory β-cell expansion during pregnancy. Blood samples were collected from fed dams at E18.5 (*A* and *F*) or during GTT after 6-h fasting (*B*). Insulin concentrations were measured using a Bio-Plex kit (Bio-Rad). *C, D, E,* and *G*: Pancreases were collected at E18.5. *C*: Islet architecture was detected by immunofluorescence staining of pancreas with insulin (green) and glucagon (red) (original magnification ×10). Islet areas were measured using anti-insulin antibody–stained sections with Leica SlidePath software (*D*). β-Cell mass was calculated using serially step-sectioned pancreatic samples (*E* and *G*). Islets were isolated by collagenase digestion and differential centrifugation (*H*). Similar-size islets were handpicked and cultured in Krebs-Ringer medium with 2 mmol/L glucose (LG), 20 mmol/L glucose (HG), or 30 mmol/L KCl for 1 h. Insulin concentrations of medium were determined. Data are presented as mean ± SEM; $n = 8$. *$P < 0.05$ vs. WT or vs. Ad-*gfp*–treated *Adipoq*$^{-/-}$ dams.

hypoadiponectinemia, which may be one of the multiply underlying mechanisms of GDM. In humans, hypoadiponectinemia is usually induced by prolonged obesity, which impairs metabolism through various pathways in addition to adiponectin. *Adipoq*$^{-/-}$ dams were not obese. Therefore, this genetic mouse model provides a tool to study the regulatory role of adiponectin in modulating maternal metabolism during pregnancy. Although their metabolic phenotypes resemble GDM, other factors such as obesity and insulin resistance are absent in *Adipoq*$^{-/-}$ dams. Insulin resistance has been detected in skeletal muscle of human subjects with GDM and has been considered an underlying mechanism of glucose intolerance in GDM (26,27). Interestingly, despite glucose intolerance in *Adipoq*$^{-/-}$ dams, insulin-stimulated glucose disposal rates and insulin signaling in metabolically active tissues were not significantly altered. Obviously, we cannot attribute insulin resistance to glucose intolerance of pregnant *Adipoq*$^{-/-}$ mice. Consistent with the studies of nonpregnant *Adipoq*$^{-/-}$ mice (30), our study indicates that adiponectin deficiency increases HGP in pregnant mice, which might contribute to increased basal blood glucose of *Adipoq*$^{-/-}$ dams. Our clamp study also revealed that although expression of rate-limiting enzymes of gluconeogenesis and basal HGP rates were remarkably elevated, insulin infusion still efficiently suppressed HGP in *Adipoq*$^{-/-}$ dams. Furthermore, our study found that phosphorylation levels of AMPK in livers of *Adipoq*$^{-/-}$ dams were similar to those of WT control dams. Therefore, our results indicate that adiponectin deficiency increases HGP in pregnant mice through a mechanism(s) independent of insulin and AMPK signaling. Similarly, Birnbaum and colleagues (31) demonstrated that adiponectin inhibits hepatic gluconeogenesis independent of AMPK. Regarding insulin signaling, adiponectin is known for its insulin-sensitizing effect. Mouse studies, including our own work, reveal that adiponectin deficiency does not alter insulin signaling when mice were fed with chow (14,35). One study even reported an inhibitory effect of adiponectin on insulin signaling in trophoblast cells (38). Due to the scope limitation, our study does not provide any experimental data to explain the lack of effect of adiponectin deficiency on insulin signaling. However, these animal studies at least indicate that the beneficial effect of adiponectin on insulin sensitivity may be through an indirect effect(s) instead of directly stimulating protein(s) in the insulin network.

Hyperlipidemia is another hallmark of GDM (39). Similar to higher blood glucose concentrations, significantly

increased blood TG and FFA in *Adipoq*$^{-/-}$ dams were only observed during pregnancy. Although many factors affect blood TG levels, high blood TG concentrations are commonly caused by increased hepatic TG production and/or decreased TG catabolism in tissues. Unlike nonpregnant mice (16), the current study found that LPL expression and activity in skeletal muscle of *Adipoq*$^{-/-}$ dams were similar to those of WT controls. Our study does not provide any data that explain the inconsistency between pregnant and nonpregnant mice. However, LPL activity in skeletal muscle is significantly reduced during normal pregnancy (Supplementary Fig. 1), which may obscure the regulatory effects of adiponectin on LPL expression during pregnancy. However, our study found that hepatic TG production rates were robustly increased in *Adipoq*$^{-/-}$ dams, which may underlie adiponectin deficiency–induced hypertriglyceridemia during pregnancy. Furthermore, similar to nonpregnant mice, increased lipolysis was found in WAT of *Adipoq*$^{-/-}$ dams. Increased WAT lipolysis should contribute to increased blood FFA but also provide substrates for hepatic TG synthesis and lipoprotein particle packing.

During pregnancy, the development of physiological insulin resistance and maternal metabolic adaptations impose a prolonged demand for insulin (33). To meet this demand, a compensatory expansion of β-cell mass occurs (33), resulting in increasing blood insulin concentrations (21). Inadequate β-cell expansion causes insulin insufficiency and GDM (40–42). Our study revealed that both β-cell mass and blood insulin concentrations were significantly decreased in *Adipoq*$^{-/-}$ dams, which indicate that maternal adiponectin deficiency induces insulin insufficiency in mice. Our study also revealed that insulin sensitivity was intact in most metabolically active tissues of *Adipoq*$^{-/-}$ dams. Therefore, insulin insufficiency should play an important role in maternal adiponectin deficiency–induced glucose intolerance and hyperlipidemia. Regarding how maternal adiponectin deficiency induces insulin insufficiency, a human study reported a strong association between maternal blood adiponectin and β-cell function in late pregnancy and suggests that hypoadiponectinemia plays a key role in mediating β-cell dysfunction and the pathogenesis of GDM (43). Other studies have reported that adiponectin enhances β-cell proliferation (44,45). Interestingly, the effects of adiponectin on insulin secretion were inconsistent in those studies (43,44,46,47). Our study observed a significant decrease in β-cell mass of *Adipoq*$^{-/-}$ dams, whereas adiponectin reconstitution restored maternal β-cell mass and blood insulin concentrations. Therefore, maternal adiponectin deficiency might impair pregnancy-induced compensatory β-cell proliferation and islet mass expansion, which together likely produced insulin insufficiency. High-molecular-weight adiponectin has been suggested as a main determinant of β-cell function in subjects with GDM (48). Since adiponectin expression was completely blocked in the *Adipoq*$^{-/-}$ mice (14), our study does not provide any clue about which multimeric adiponectin enhances compensatory β-cell proliferation during pregnancy.

In summary, using a genetic mouse model, our study demonstrates that maternal adiponectin deficiency induces spontaneous development of the main characteristics of GDM, including glucose intolerance, hyperlipidemia, and enhancement of fetal growth. Our study also provides evidence indicating that insulin insufficiency, enhanced WAT lipolysis, and hepatic glucose and TG production mediate adiponectin deficiency–impaired metabolism in pregnant mice. Further studies are required to dissect the relationship between β-cell expansion and direct effects on metabolically active tissues in adiponectin-controlled maternal metabolic adaptation to pregnancy.

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